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Short communication

Intraventricular insulin reduces the antinociceptive effect of [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin in mice

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Abstract

The effects of pretreatment with insulin on the antinociception induced by intracerebroventricular (i.c.v.) administration of the μ-opioid receptor agonist [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO) were studied in mice. Intracerebroventricular pretreatment with insulin (1 and 3 mU) for 60 min dose dependently attenuated the antinociception induced by i.c.v. DAMGO (5.6 ng) in mice. Intracerebroventricular pretreatment with a highly selective tyrosine kinase inhibitor, lavendustin A, at doses of 100 and 300 ng for 10 min, dose dependently reversed the antinociceptive effect of DAMGO (5.6 ng) in insulin-treated mice. The antinociceptive effect of DAMGO (5.6 ng, i.c.v.) was significantly reduced in C57BL/KsJ-db/db diabetic mice compared with that in age-matched control (C57BL/KsJ-db/++) mice. When C57BL/KsJ-db/db diabetic mice were pretreated with lavendustin A (300 ng), the antinociceptive effect of DAMGO was significantly increased. These results indicate that tyrosine kinase may be involved in the reduction of DAMGO-induced antinociception by insulin in mice. Furthermore, the attenuation of DAMGO-induced antinociception in C57BL/KsJ-db/db diabetic mice may be due in part to increased tyrosine kinase activity as a result of hyperinsulinemia. © 1998 Elsevier Science B.V.

Keywords: Antinociception; μ-Opioid receptor; Protein tyrosine kinase; Lavendustin A; Protein kinase C; C57BL/KsJ-db/db mice; Diabetes

1. Introduction

It has been reported that the antinociceptive potency of morphine is decreased in spontaneously diabetic (C57BL/KsJ-db/db) mice, an animal model of non-insulin-dependent diabetes mellitus (type II diabetes) (Simon and Dewey, 1981). In this regard, we recently reported that (±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy)benzyl]-2,4-thiazolidinedione (CS-045), an oral antidiabetic agent, dramatically improved the antinociceptive effect of morphine in C57BL/KsJ-db/db mice (Kamei et al., 1997). In contrast to the hyperglycemia and hypoinsulinemia observed in streptozotocin-induced diabetic mice, spontaneously diabetic mice (C57BL/KsJ-db/db) are hyperglycemic and hyperinsulinemic (Coleman and Hummel, 1967). CS-045 decreases plasma glucose and insulin levels in spontaneously diabetic mice (Fujiwara et al., 1988, 1991). Therefore, it seems likely that not only hyperglycemia but also hyperinsulinemia may be responsible for the reduction in the antinociceptive effects of μ-opioid receptor agonists in C57BL/KsJ-db/db mice. However, there is no information available regarding the effect of insulin on the antinociceptive effects of μ-opioid receptor agonists.

Thus, the first aim of the present study was to investigate the effect of i.c.v. insulin on the antinociceptive effect of [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO) to clarify the hypothesis that hyperinsulinemia may be responsible for the reduction in the antinociceptive effects of μ-opioid receptor agonists. We also examined the effect of lavendustin A, a specific inhibitor of tyrosine kinase (O'Dell et al., 1991, Onoda et al., 1989), on the antinociceptive effect of DAMGO in C57BL/KsJ-db/db mice, because insulin has been reported to activate phosphorylation of the receptor protein tyrosine kinase.

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2. Materials and methods

2.1. Animals

Male C57BL/KsJ diabetic (db/db) mice and C57BL/KsJ non-diabetic (db/++) mice were generously supplied by Dr. Fujiwara (Institute of Experimental Animals, Tokyo University, School of Medicine, Tokyo, Japan) and maintained at Yokohama City University, School of Medicine. C57BL/KsJ-db/db mice were used for the experiments at 20–24 weeks of age. Male 6-week-old ICR mice weighing about 25 g were purchased from Tokyo Animal Laboratory, Tokyo, Japan. They had free access to food and water in an animal room which was maintained at 24 ± 1°C with a 12-h light/dark cycle. These studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Assay of the extent of antinociception

The antinociceptive response was evaluated in the tail-flick test. The intensity of a heat lamp was adjusted to provide a pre-drug latency time in the tail-flick response of 2–4 s. A cut-off latency of 15 s was used to prevent injury to the tail. Animals that did not respond within 15 s were removed and assigned a score of 15 s. The percent maximum possible effect (%MPE) was calculated for each animal according to the formula: %MPE = 100 × (post-drug latency-pre-drug latency)/(15-pre-drug latency). Data are expressed as means ± S.E.

2.3. Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed following the method described by Haley and McCormick (1957), using a 50-μl Hamilton syringe. The injection site was 1.5 mm from the midline, 0 mm from the bregma and 3.0 mm from the surface of the skull. Injection volumes were 5 μl for i.c.v. administration.

2.4. Drugs

The following drugs were used: (D-Ala^2, *N*-MePhe^4, Gly-ol^5) enkephalin (DAMGO; Peninsula Laboratories, San Carlos, CA, USA), lavendustin A (Calbiochem-Novabiochem, San Diego, CA, USA), porcine insulin (Biomedical Technologies, Stoughton, MA, USA). Lavendustin A was dissolved in 0.6% dimethyl sulfoxide DMSO in saline (0.9% NaCl). DAMGO and insulin were dissolved in saline. Antinociceptive effects were assessed 10, 20, 30 and 60 min after i.c.v. administration of DAMGO. Insulin and lavendustin A were administered 60 min before DAMGO treatment.

2.5. Data analysis

The data are expressed as means ± S.E. The statistical significance of differences between groups was assessed with Student's *t*-test (comparison of two groups) or an analysis of variance (ANOVA) followed by the Bonferroni test (comparisons among multiple groups).

3. Results

3.1. Effects of insulin on the antinociceptive effect of DAMGO

Intracerebroventricular administration of DAMGO (5.6 ng) produced an average %MPE of 63.4 ± 9.3% in mice. As shown in Fig. 1A, pretreatment with insulin (1 and 3 mU, i.c.v.) 60 min prior to i.c.v. challenge with DAMGO dose dependently attenuated the antinociceptive effect of DAMGO (5.6 ng). Furthermore, pretreatment with insulin (3 mU, i.c.v.) produced a 2.1-fold rightward shift in the dose-response curve for DAMGO-induced antinociception (Fig. 1B).

The effects of lavendustin A on the antinociceptive effect of DAMGO in insulin-pretreated mice are shown in Fig. 1C. Pretreatment with lavendustin A for 60 min, at doses of 100 and 300 ng, i.c.v., dose dependently reversed the insulin-induced suppression of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.).

However, insulin and lavendustin A, by themselves, had no significant effect on the tail-flick latency (insulin: pre-drug value, 2.4 ± 0.1 s, post-drug value, 2.5 ± 0.2 s, $n = 10$; lavendustin A, 100 ng: pre-drug value, 2.3 ± 0.2 s, post-drug value, 2.4 ± 0.1 s, $n = 10$; lavendustin A, 300 ng: pre-drug value, 2.4 ± 0.1 s, post-drug value, 2.6 ± 0.1 s, $n = 10$).

3.2. Effect of lavendustin A on the antinociceptive effect of DAMGO in C57BL / KsJ-db / db mice

The time courses of the antinociceptive effects of i.c.v. administration of DAMGO (5.6 ng) in C57BL/KsJ-db/db diabetic mice and C57BL/KsJ-db/++ non-diabetic mice are shown in Fig. 2. The antinociceptive effect of DAMGO was significantly reduced in C57BL/KsJ-db/db mice compared to that in age-matched C57BL/KsJ-db/++ non-diabetic mice. When lavendustin A (300 ng, i.c.v.) was administered to C57BL/KsJ-db/db mice, DAMGO produced a pronounced increase in the tail-flick latency. Indeed, the antinociceptive effect of DAMGO in lavendustin A-treated C57BL/KsJ-db/db mice was significantly increased compared with that in lavendustin A-untreated C57BL/KsJ-db/db mice (Fig. 2). However, the

antinociceptive effect of DAMGO in lavendustin A-treated C57BL/KsJ-db/db mice was still significantly reduced compared with that in C57BL/KsJ-db/++ mice. Lavendustin A had no significant effect on the antinociceptive effect of DAMGO in C57BL/KsJ-db/++ mice. Furthermore, lavendustin A (300 ng, i.c.v.), by itself, had no significant effect on the tail-flick latency in either C57BL/KsJ-db/db (pre-drug value, 2.6 ± 0.1 s, post-drug value, 2.5 ± 0.1 s, $n = 8$) or C57BL/KsJ-db/++ mice (pre-drug value, 2.4 ± 0.2 s, post-drug value, 2.5 ± 0.2 s, $n = 7$).

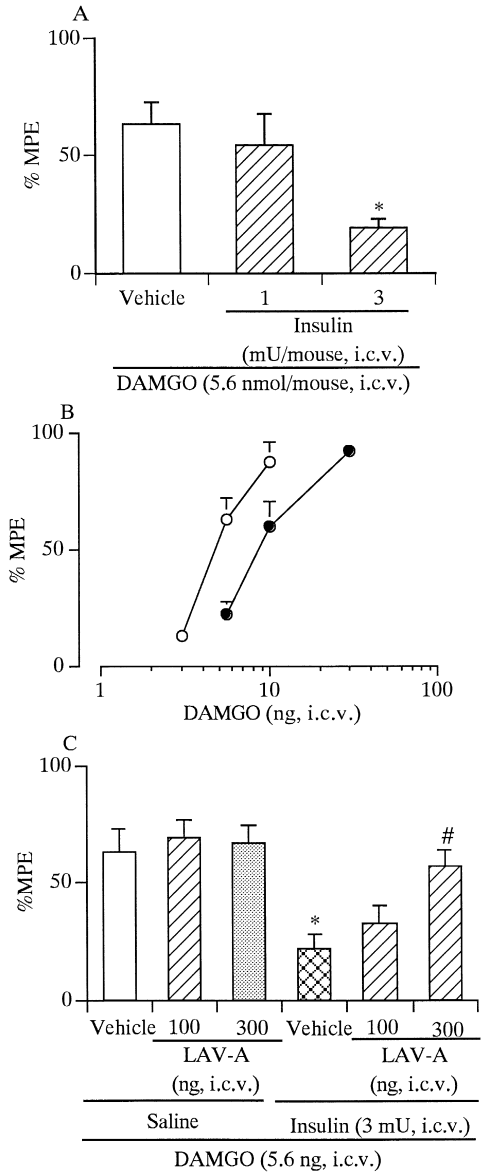

Fig. 1. (A) Effect of insulin on DAMGO (5.6 ng, i.c.v.)-induced antinociception in mice. (B) Effect of i.c.v. pretreatment with insulin (3 mU, i.c.v.) on the dose–response curve for DAMGO-induced antinociception. (C) Effects of lavendustin A (LAV-A, 100 and 300 ng, i.c.v.) on the insulin (3 mU, i.c.v.)-induced reduction of DAMGO (5.6 ng, i.c.v.)-induced antinociception. Insulin and lavendustin A were injected i.c.v. 60 min before the administration of DAMGO. Mice were tested 10 min after the injection of DAMGO. Each point represents the mean with S.E. for 10–12 animals. $* P < 0.05$ vs. respective saline-treated group. $\#P < 0.05$ vs. respective vehicle (0.6% DMSO)-treated group.

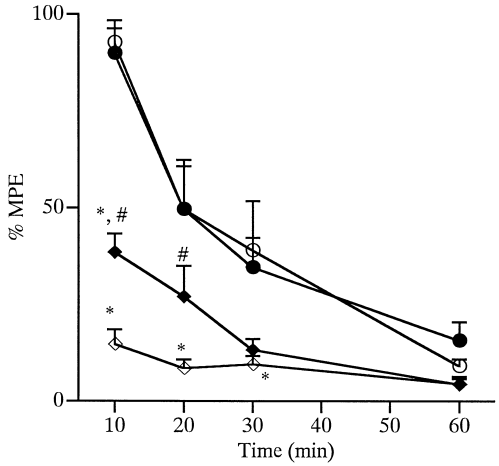

Fig. 2. Effect of lavendustin A on the antinociceptive effect of DAMGO (5.6 ng, i.c.v.) in C57BL/KsJ-db/db (diamond) and C57BL/KsJ-db/++ (circle) mice. Lavendustin A (300 ng, i.c.v., closed symbol) and vehicle (0.6% DMSO, open symbol) were injected i.c.v. 60 min before the administration of DAMGO. Mice were tested 10, 20, 30 and 60 min after the injection of DAMGO. Each point represents the mean with S.E. for 7–8 animals. $* P < 0.05$ vs. respective C57BL/KsJ-db/++ mice. $\#P < 0.05$ vs. vehicle-treated C57BL/KsJ-db/db mice.

4. Discussion

The results of this study demonstrate that i.c.v. pretreatment with insulin (3 mU) attenuated the inhibition of the tail-flick response induced by i.c.v. DAMGO in mice. Furthermore, this attenuation of i.c.v. DAMGO-induced antinociception by insulin was reversed by i.c.v. pretreatment with lavendustin A. Onoda et al. (1989) and O'Dell et al. (1991) demonstrated that the inhibitory effects of lavendustin A on serine and threonine kinase, such as protein kinase C, protein kinase A and Ca^{2+}/calmodulin protein kinase, are much less than those on tyrosine kinase, but not other kinases. It is possible that the effect of lavendustin A observed in the present study is related to tyrosine kinase, but not other kinases. These results suggest that the attenuation by insulin of DAMGO-induced antinociception is specifically mediated by the activation of tyrosine kinase.

In the present study, we also observed that pretreatment with lavendustin A (300 ng, i.c.v.), which had no significant effect on DAMGO-induced antinociception in C57BL/KsJ-db/++ non-diabetic mice, significantly increased DAMGO-induced antinociception in C57BL/KsJ-db/db diabetic mice. These results suggest that the activation of tyrosine kinase may be involved in the reduction of the antinociceptive effect of DAMGO in C57BL/KsJ-db/db mice. We recently reported that CS-045, an oral antidiabetic agent, administered to C57BL/KsJ-db/db diabetic mice completely reversed the antinociceptive effect of morphine to the levels observed in C57BL/KsJ-

db/++ mice. Although lavendustin A significantly increased the antinociceptive effect of DAMGO in C57BL/KsJ-db/db mice, the antinociceptive effect of DAMGO in C57BL/KsJ-db/db was still significantly lower than that in C57BL/KsJ-db/++ mice. CS-045 decreased not only serum glucose levels, but also insulin levels in C57BL/KsJ-db/db mice. Since the sensitivity to the antinociceptive effect of morphine returned to normal when insulin was administered to streptozotocin-induced diabetic mice to lower their serum glucose levels, it has been suggested that diabetes-induced hyperglycemia is responsible for the reduction of the antinociceptive effect of μ-opioid receptor agonists (Simon and Dewey, 1981; Kamei et al., 1993). Thus, it is possible that not only hyperinsulinemia-induced activation of tyrosine kinase but also hyperglycemia may be involved in the reduction in DAMGO-induced antinociception in C57BL/KsJ-db/db mice.

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